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Enhancing Effects of Quinacrine on Development of Hepatopancreatic Lesions in *N*-Nitrosobis(2-oxopropyl)amine-initiated Hamsters

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The modifying effects of quinacrine administration during the post-initiation phase of carcinogenesis were investigated in hamsters treated with *N*-nitrosobis(2-oxopropyl)amine (BOP). Female Syrian hamsters were given three weekly s.c. injections of BOP at a dose of 10 mg/kg and then 300 or 100 ppm quinacrine in their diet for 37 weeks. Additional groups of animals received the BOP injection alone, or only the 300 ppm quinacrine treatment as BOP-negative controls. At week 40 of the experiment, all surviving animals were killed and development of proliferative lesions was assessed histopathologically. The multiplicity of pancreatic adenocarcinomas and dysplastic lesions per hamster was significantly higher ($P<0.01$ and $P<0.05$) in the BOP/Q100 group (1.92 and 1.78) than in the BOP-alone group (1.07 and 0.79). The incidence of hepatocellular adenomas plus carcinomas was also significantly elevated ($P<0.05$) in the BOP/Q300 and BOP/Q100 groups. In contrast, the multiplicity of lung adenomas plus adenocarcinomas was significantly decreased ($P<0.05$) by the Q300 treatment. Neither the incidence nor the multiplicity of renal cell tumors (adenomas and carcinomas) or nephroblastomas significantly differed between the BOP-treated groups. Electron microscopic examination revealed an abundance of myeloid lamellar bodies filling the cytoplasm of hepatocytes and pancreatic ductular and acinar cells, and epithelial cells of the gallbladder in the quinacrine-treated animals, the degree being dose-dependent. Our results indicate that quinacrine enhances pancreatic and hepatic carcinogenesis in hamsters induced by BOP.

Key words: Quinacrine — Pancreatic carcinogenesis — Hamster — BOP

Quinacrine (6-chloro-9-[[4-(diethylamino)-1-methylbutyl]amino]-2-methoxyacridine; CAS no. 83-90-6) is an acridine derivative that has been used as an antimalarial and anthelminthic agent.[1)] This drug has an amphophilic chemical structure and accumulates in cytoplasmic lysosomes, forming complexes with phospholipids. Its chronic administration results in generalized phospholipidosis in animals, involving various organs such as the liver, kidney, lung, spleen, lymph node, adrenal and thyroid.[2, 3)] A similar phospholipidosis can be induced with 4-4′-diethylaminoethoxyhexestrol, a coronary dilator reported to cause hepatosplenomegaly and phospholipid fatty liver in humans, with myelin-like structures being observed under the electron microscope.[4)] Quinacrine has been reported to cause liver dysfunction, and also to induce hepatic injury in a patient after prolonged treatment,[5)] although a protective action of quinacrine in carbon tetrachloride-induced liver necrosis has also been shown.[6)] It shows marginal mutagenicity in *Salmonella typhimurium*,[7)] and since its administration results in lysosomal dysfunction, enzymatic alterations in hepatocytes and liver cell damage, it might be expected to exert modifying effects on hepatocarcinogenesis, although no carcinogenicity data have yet been reported. In fact, it dose-dependently enhanced the development of glutathione *S*-transferase placental form (GST-P)-positive liver cell foci in a rapid *in vivo* rat liver bioassay system, associated with the presence of abundant myeloid lamellar bodies filling the cytoplasm of hepatocytes and bile duct epithelial cells.[8)]

N-Nitrosobis(2-oxopropyl)amine (BOP) has been shown to induce lung, pancreatic, liver and kidney tumors in hamsters.[9, 10)] It is considered to be particularly advantageous for assessing the modifying effects of chemicals on pancreatic and lung carcinogenicity because of the histological and biological similarities of the induced lesions to those observed in man.[11)] The present experiment was performed to elucidate the effects of quinacrine during the post-initiation phase in the BOP-induced carcinogenesis model in hamsters.

MATERIALS AND METHODS

Animals and chemicals A total of 120 female Syrian hamsters (Japan SLC, Inc., Shizuoka), 5 weeks old and weighing about 80 g at the commencement, were used in this experiment. The animals were housed, five per polycarbonate cage, in an air-conditioned room at 23±2°C and 60±5% humidity under a daily cycle of alternating 12-h period of light and darkness. Oriental MF powder diet (Oriental Yeast Co., Ltd., Tokyo) and tap water were available *ad libitum*. BOP was obtained from Nacalai Tesque (Kyoto) and quinacrine from Tokyo Kasei Kogyo Co., Ltd. (Tokyo).

Experimental protocol As shown in Fig. 1, groups 1–3, each consisting of 30 hamsters, were given BOP subcutaneously once a week for 3 weeks at a dose of 10 mg/kg

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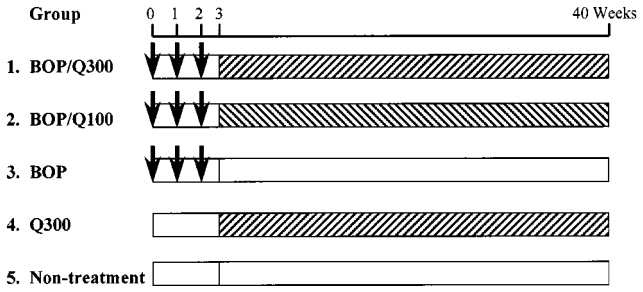

Fig. 1. Experimental design. ↓ *N*-nitrosobis(2-oxopropyl)amine (BOP) 10 mg/kg body weight, s.c.; ▨ 300 ppm quinacrine in diet; ▨ 100 ppm quinacrine in diet.

body wt. After this initiation treatment, the animals were continuously fed diet supplemented with 300 ppm (group 1) or 100 ppm (group 2) quinacrine, or no supplement (group 3), during the post-initiation stage (weeks 3–40). Groups 4 and 5, each consisting of 10 hamsters, received 300 ppm quinacrine and basal diet alone, respectively, thus serving as initiation-negative controls. The doses of quinacrine used in the present study were selected on the basis of previous experimental results for rats.[8] The hamsters were observed daily and weighed once every 4 weeks. At the end of week 40, all surviving animals were killed and examined. Moribund or dead animals were also completely autopsied for histological examination.

Histological examination At autopsy, the pancreas, lung, liver and kidney were carefully examined macroscopically, and then fixed in 10% phosphate-buffered formalin for conventional embedding, and observation of sections stained with hematoxylin and eosin. Proliferative lesions were diagnosed histopathologically and counted in representative sections. Histological criteria for bile duct hyperplasia, cholangiofibrosis, cholangioma and carcinoma of the liver were based on the classification by Eustis *et al*.[12] For electron microscopic examination, samples of pancreas and liver tissues from the 300 ppm quinacrine-treated groups were fixed immediately in 2.5% glutaraldehyde, post-fixed with 1% osmium tetroxide, then embedded in epoxy resin, and observed under a JEM-1200EX electron microscope (JEOL, Tokyo).

The results were statistically analyzed by analysis of variance (ANOVA) followed by Fisher's exact probability test.

RESULTS

Body and organ weights Final body weight gains were significantly increased in the BOP/Q100 ($P<0.01$), BOP-alone ($P<0.05$) and Q300 groups ($P<0.01$) as compared to the non-treatment group (Table I). Absolute liver weights were significantly increased in the BOP/Q300 ($P<0.01$), BOP/Q100 ($P<0.01$), BOP-alone ($P<0.05$) and Q300 groups ($P<0.01$) as compared to the non-treatment group (Table I). Relative liver weights were significantly increased in the BOP/Q300 ($P<0.01$), BOP/Q100 ($P<0.01$) and Q300 groups ($P<0.01$) as compared to the non-treatment group (Table I). There were no statistically significant differences between the groups given BOP plus quinacrine and quinacrine alone. Death rates of hamsters in the BOP/Q300, BOP/Q100, BOP-alone, Q300 and non-

Table I. Body and Liver Weights

Group	Finalbody weight (g)	Liver weight (g)	Relative liver weight (%)
1. BOP/Q300	166.7±27.4[a)]	7.71±1.34[##]	4.74±1.19[##]
2. BOP/Q100	172.4±17.8[##]	8.90±1.52*,[##]	5.23±1.10[##]
3. BOP	170.1±23.9[#]	7.38±3.33[#]	4.57±2.72
4. Q300	188.4±18.1[##]	8.11±1.14[##]	4.34±0.69[##]
5. Non-treatment	152.8±14.7	5.52±0.99	3.57±0.72

a) Mean±SD.
* $P<0.05$ compared with the BOP-alone group. # $P<0.05$, ## $P<0.01$ compared with the non-treatment group.

Table II. Incidences and Multiplicity of Pancreatic Proliferative Lesions

Group	Effective no. of animals	No. of animals with			No. of tumors/animal		
		Adc[a)]	Dys	Total (%)	Adc	Dys	Total
1. BOP/Q300	30	23	14	27 (90.0)	1.53±1.27[b)]	1.06±1.31	2.60±1.83
2. BOP/Q100	28	26	20	28 (100)	1.92±1.30**	1.78±1.72*	3.71±2.05**
3. BOP	29	22	15	24 (82.7)	1.07±0.80	0.79±1.01	1.86±1.48
4. Q300	20	0	0	0	0	0	0
5. Non-treatment	10	0	0	0	0	0	0

a) Adc, adenocarcinoma; Dys, dysplastic lesion.
b) Mean±SD.
* $P<0.05$, ** $P<0.01$ compared with the BOP-alone group.

Table III. Incidences and Multiplicity of Liver Tumors

Group	Hepatocellular			Cholangiocellular		
	Adenoma	Carcinoma	Total	Adenoma	Carcinoma	Total
BOP/Q300	0.17±0.60[a)]	0.20±0.49	0.36±0.94	0.10±0.30	0.17±0.38	0.27±0.45
	10.3[b)]	17.2	24.1*	10.3	17.2	27.5
BOP/Q100	0.31±0.71*	0.20±0.61	0.51±1.05*	0.17±0.38	0.24±0.43	0.41±0.50
	20.6*	13.7	34.4**	17.2	24.1	41.3
BOP	0	0.04±0.20	0.04±0.20	0.20±0.50	0.32±0.47	0.52±0.71
	0.0	4.0	4.0	16.0	32.0	48.0

a) Mean±SD, multiplicity data.
b) Percentage incidence data.
∗ $P<0.05$, ∗∗ $P<0.01$ compared with the BOP-alone group.

Table IV. Incidence and Multiplicity of Lung and Kidney Tumors

Group	Lung			Kidney			
	Ad[a)]	Adc	Total	Ad	Adc	Total	Blastoma
BOP/Q300	1.21±1.34[b)]	0.28±0.65	1.50±1.52	0.07±0.26	0.03±0.19	0.11±0.32	0
	57.1[c)]	17.8	64.2*	27.1	3.5	10.7	0.0
BOP/Q100	1.60±1.81	0.71±0.80	2.32±2.10	0	0.03±0.18	0.04±0.19	0.07±0.26
	64.2	50.0	75.0	0.0	3.5	3.5	7.1
BOP	1.37±1.43	0.52±0.77	1.91±1.88	0	0	0	0
	75.0	41.6	91.6	0.0	0.0	0.0	0.0

a) Ad, Adenoma; Adc, adenocarcinoma.
b) Mean±SD, multiplicity data.
c) Percentage incidence data.
∗ $P<0.05$ compared with the BOP-alone group.

treatment groups were 16.7%, 23.4%, 40.0%, 10.0% and 10.0%, respectively.

Effects of quinacrine on pancreatic carcinogenesis Incidence and multiplicity data for histopathologically diagnosed pancreatic lesions observed in each group of hamsters are summarized in Table II. The incidences of pancreatic adenocarcinomas and dysplastic lesions were not statistically significantly different among the BOP-treated groups, although quinacrine administration was associated with a tendency for increase. However, the multiplicities of both adenocarcinomas and dysplastic lesions per hamster were significantly higher ($P<0.01$ and $P<0.05$) in the BOP/Q100 group (1.92 and 1.78) than in the BOP-alone group (1.07 and 0.79). The total multiplicity of combined adenocarcinomas and dysplastic lesions was also significantly increased ($P<0.01$) in the BOP/Q100 group (3.71) as compared to the BOP-alone case (1.86).

Atrophic or fatty changes of pancreatic exocrine tissues were more severe in the Q300 group than in the non-treatment group.

Effects of quinacrine on hepatocarcinogenesis As indicated in Table III, the incidences of hepatocellular adenomas plus carcinomas were significantly higher ($P<0.05$) in the BOP/Q300 and BOP/Q100 groups than in the BOP group. The multiplicities of hepatocellular adenomas and total hepatocellular tumors were also significantly elevated ($P<0.05$) by the BOP/Q100 treatment. No intergroup variation was noted for cholangiomas and cholangiocarcinomas after BOP treatment.

Hepatocytes of quinacrine-treated groups, regardless of BOP treatment, were enlarged in association with a ground-glass appearance and yellowish pigmentation of the cytoplasm, although no necrosis was apparent at the termination of the experiment. The BOP treatment groups showed bile duct hyperplasias and cholangiofibrosis in the liver, and epithelial hyperplasias in the gallbladder.

Although quinacrine administration was associated with a tendency for increase in gallbladder epithelial hyperplasia, no tumors developed in the gallbladder in the present study.

Effects of quinacrine on lung carcinogenesis The multiplicity of adenomas plus adenocarcinomas was significantly smaller ($P<0.05$) in the BOP/Q300 group as

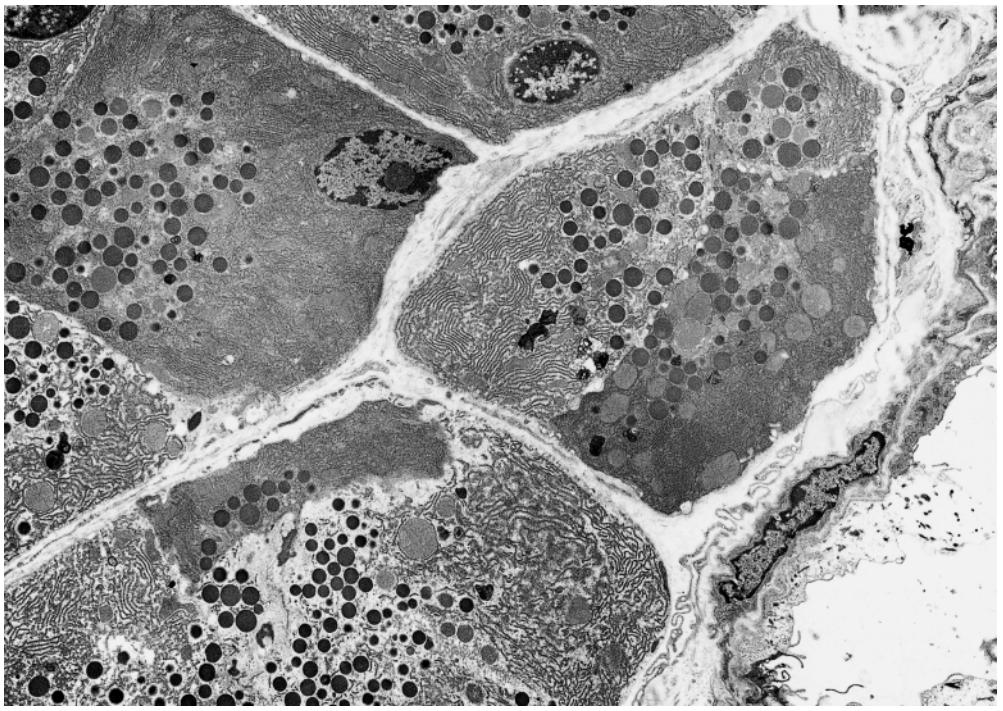

Fig. 2. Electron microscopic appearance of pancreatic acini in a 300 ppm quinacrine-treated hamster. Note the myeloid lamella bodies in the cytoplasm. ×3000.

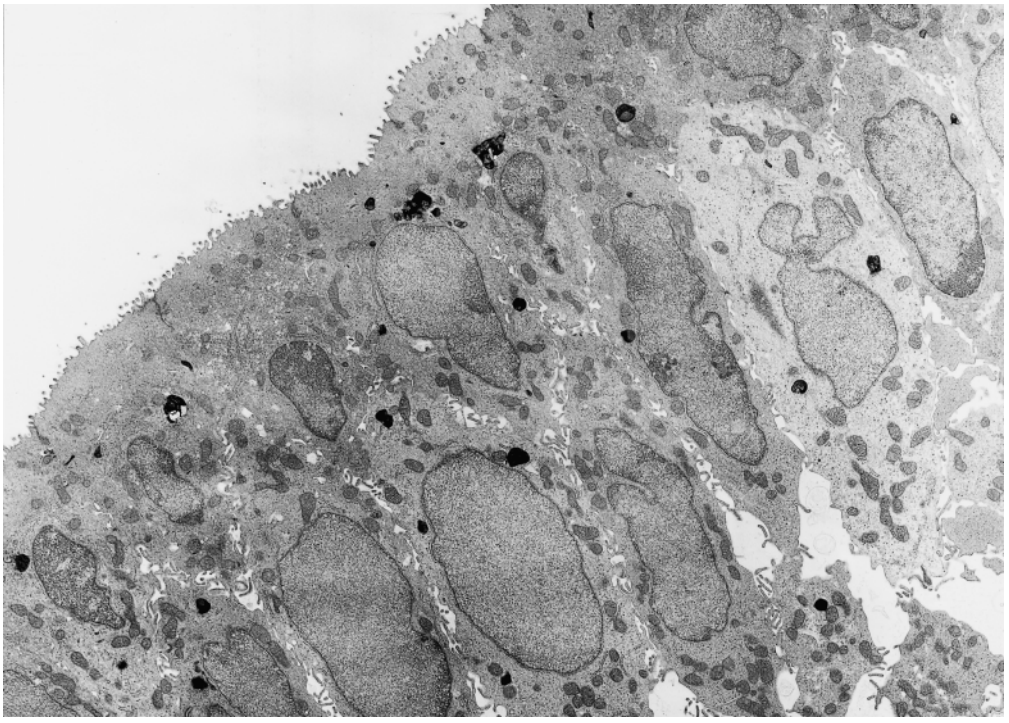

Fig. 3. Gallbladder mucosal epithelium from a 300 ppm quinacrine-treated hamster. Myeloid lameller bodies are apparent. ×3700.

compared to the BOP-alone group, although the incidences did not differ among the BOP-treated groups (Table IV).

Effects of quinacrine on renal carcinogenesis Neither the incidence nor the multiplicity of renal cell tumors (adenomas and carcinomas) or nephroblastomas was significantly affected by quinacrine treatment, although a tendency for increase in renal cell tumors was noted (Table IV).

Electron microscopic examination An abundance of myeloid lamellar bodies in the cytoplasm of hepatocytes was observed in all quinacrine-treated groups, including that receiving the 300 ppm dose. Such inclusion bodies were also observed to a lesser extent in cells of the pancreatic duct epithelium and acinus (Fig. 2), and the gallbladder mucosal epithelium of these hamsters (Fig. 3), although no pancreatic acinar or gallbladder tumors developed in the present study.

DISCUSSION

The results of the present study demonstrate that quinacrine enhances the development of proliferative lesions in the pancreas and liver of hamsters initiated with BOP. Therefore, our findings are in line with those obtained with a rapid *in vivo* rat liver bioassay system, showing a potential promotion effect on hepatocarcinogenesis.[8] Quinacrine induces phospholipidosis in hepatocytes and bile duct epithelial cells of rats, with generation of myeloid lamellar cytoplasmic inclusion bodies.[8] The present ultrastructural study also showed an abundance of myeloid lamellar bodies in the cytoplasm of hepatocytes as well as to a lesser extent in pancreatic duct epithelial cells, acinar cells and gallbladder epithelial cells in the quinacrine-treated groups, regardless of the BOP treatment. Therefore, similar mechanisms could underlie the promoting effects of quinacrine in both cases, although accumulation of myeloid lamellar bodies does not by itself explain the promotive effects of quinacrine.

The presence of atrophic changes of pancreatic exocrine tissue in the Q300 group is clear evidence of pancreatic toxicity. In an earlier study we found that soybean trypsin inhibitor (SBTI) treatment inhibited hamster pancreatic ductal carcinogenesis when given in the phase after initiation with BOP,[13] associated with protection against atrophic changes. Thus a link with proliferation may exist. Myeloid lamellar cytoplasmic inclusion bodies in the pancreas are thought to play a role in the development of acinar cell necrosis, being related to phospholipidosis.[2, 3] Such ultrastructural changes have been noted in both experimental animals and humans administered cationic amphophilic drugs.[4, 5] Although the biological characteristics of these inclusion bodies in acinar cells remains unclear, they do appear to be associated with dysfunction. Necrosis and regenerative proliferation of acinar cells could clearly act as a promoting stimulus for pancreatic carcinogenesis. The effects of quinacrine in the liver and pancreas seem different because growth promotion was seen in the liver, but atrophy in the pancreas. It is likely that, after injury, cell proliferation of hepatocytes is faster than that of pancreatic acinar cells, with atrophic changes preferentially occurring in pancreatic acinar cells. Lack of dose-dependency of the enhancing effects on pancreatic ductal and hepatocellular lesions might be related to the severity of cytotoxicity of quinacrine.

Quinacrine is also known to be a relatively specific phospholipase A_2 inhibitor,[14–16] which restricts arachidonic acid release from membrane phospholipids, eventually reducing the production of eicosanoids (prostaglandins and leukotrienes). Prostaglandin synthesis inhibitors have been shown to modulate mammary gland carcinogenesis[17, 18] and pancreatic carcinogenesis in hamsters after initiation with BOP.[19] Quinacrine may in fact inhibit mammary tumor development for this reason[20] but clearly, any such effect on the hepatopancreatic axis in the hamster was countered by enhancing effects.

In the present study, quinacrine did show inhibitory effects on lung tumor development. Myeloid lamella inclusion bodies have been reported in the lungs of rats given quinacrine[8] although the lungs were not ultrastructurally investigated in the present study. The possibility that prostaglandin synthesis inhibition or some other process may have been more pronounced than any adverse effects in this case warrants further attention.

In conclusion, our data indicate that quinacrine enhances pancreatic and hepatic carcinogenesis while inhibiting lung tumor development in hamsters initiated with BOP.

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REFERENCES

1) Rollo, I. Miscellaneous drugs used in the treatment of protozoal infections. *In* "The Pharmacological Basis of Therapeutics," ed. A. G. Gilman, L. S. Goodman and A. Gilman, pp. 1070–1079 (1980). Macmillan, New York.
2) Lüllman, H., Lüllman-Rauch, R. and Wassermann, O. Lipidosis induced by amphophilic cationic drugs. *Biochem. Pharmacol.*, **27**, 1103–1108 (1978) .
3) De Duve, C., De Barsy, T., Poole, B., Trouet, A., Tulkens,

P. and Van Hoof, F. Lysosomatopic agents. *Biochem. Pharmacol*., **23**, 2495–2531 (1974).
4) Shikata, T., Kanetaka, T., Endo, Y. and Nagashima, K. Drug-induced generalized phospholipidosis. *Acta Pathol. Jpn*., **22**, 517–531 (1972) .
5) Gibb, W., Isenberg, D. and Snait, M. I. Mepacrine-induced hepatitis. *Ann. Rheum. Dis*., **44**, 86 (1985).
6) Padrón, A. G., Toranzo, E. G. D. and Castro, J. A. Late preventive effects of quinacrine on carbon tetrachloride induced liver necrosis. *Arch. Toxicol*., **67**, 386–391 (1993).
7) Cook, T. M. and Goldman, C. K. Hycanthone and its congeners as bacterial mutagens. *J. Bacteriol*., **122**, 549–556 (1975).
8) Imaida, K., Yoshida, J., Uneyama, C., Ogasawara, H., Imazawa, T. and Hayashi, Y. Dose-dependent enhancing effects of quinacrine on induction of preneoplastic glutathione *S*-transferase placental form positive liver cell foci in male F344 rats. *Carcinogenesis*, **12**, 1911–1915 (1991).
9) Pour, P., Althoff, J., Krüger, F. W. and Mohr, U. A potent pancreatic carcinogen in Syrian golden hamsters: *N*-nitrosobis(2-oxopropyl)amine. *J. Natl. Cancer Inst*., **58**, 1449–1453 (1977).
10) Pour, P. M., Salmasi, S. Z. and Runge, R. G. Selective induction of pancreatic ductular tumors by single doses of *N*-nitrosobis(2-oxopropyl)amine in Syrian golden hamsters. *Cancer Lett*., **4**, 317–323 (1978).
11) Montesano, R., Saffiotti, U. and Shubik, P. The role of topical and systemic factors in experimental respiratory carcinogenesis. *In* "Inhalation Carcinogenesis," ed. M. G. Hanna, Jr., P. Nettesheim and J. R. Gilbert, AEC Symposium Series Vol. 18, pp. 353–371 (1970). Oak Ridge, Tennessee.
12) Eustis, S. L., Boorman, G. A., Harada, T. and Popp, J. A. Liver. *In* "Pathology of the Fischer Rat," ed. G. A. Boorman, S. L. Eustis, M. R. Elwell, C. A. Montgomery, Jr. and W. F. Mackenzie, pp. 71–94 (1990). Academic Press, San Diego.
13) Furukawa, F., Nishikawa, A., Imaida, K., Mitsui, M., Enami, T., Hayashi, Y. and Takahashi, M. Inhibitory effects of crude soybean trypsin inhibitor on pancreatic ductal carcinogenesis in hamsters after initiation with *N*-nitrosobis(2-oxopropyl)amine. *Carcinogenesis*, **13**, 2133–2135 (1992).
14) Flower, R. J. and Blackwell, G. J. The importance of phospholipase A_2 in prostaglandin biosynthesis. *Biochem. Pharmacol*., **25**, 285–291 (1976).
15) Markus, H. B. and Ball, E. G. Inhibition of lipolytic processes in rat adipose tissue by antimalarial drugs. *Biochim. Biophys. Acta*, **187**, 486–491 (1969).
16) Yorio, T. and Bentley, P. J. Phospholipase A and the mechanism of action of aldosterone. *Nature*, **271**, 79–81 (1978).
17) McCormick, D. L., Madigan, M. J. and Moon, R. C. Modulation of rat mammary carcinogenesis by indomethacin. *Cancer Res*., **45**, 1803–1808 (1985).
18) McCormick, D. L. and Moon, R. C. Inhibition of mammary carcinogenesis by flurbiprofen, a non-steroidal anti-inflammatory agent. *Br. J. Cancer*, **48**, 859–861 (1983).
19) Takahashi, M., Furukawa, F., Toyoda, K., Sato, H., Hasegawa, R., Imaida, K. and Hayashi, Y. Effects of various prostaglandin synthesis inhibitors on pancreatic carcinogenesis in hamsters after initiation with *N*-nitrosobis(2-oxopropyl)amine. *Carcinogenesis*, **11**, 393–395 (1990).
20) McCormick, D. L. Anticarcinogenic activity of quinacrine in the rat mammary gland. *Carcinogenesis*, **9**, 175–178 (1988).